SUPPLEMENTARY MATERIAL

Assessment of Ibrutinib Scheduling on Leukocyte, Lymph Node Size and Blood Pressure Dynamics in Chronic Lymphocytic Leukemia through Pharmacokinetic-Pharmacodynamic Models

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1- Dataset for model building

This data analysis included 120 patients. In all patients, ibrutinib was administrated 30 min before or 2 h after meal, except for 16 patients who were randomly assigned to receive ibrutinib either fasting or after a high-fat meal on two occasions (days 8 and 15) in a cross-over design. The patients were followed-up for a maximum of 2.4 years (median=1.7 years). However, there were periods of treatment interruptions or dose reductions due to adverse events. The dataset included 2374 ibrutinib plasma concentrations, 2434 leukocyte counts, 507 SPD and 2413 sBP and dBP blood pressure measurements. A total of 11 patients died during the study. A total of 22 patients dropped-out before the end of the study period for different reasons including disease progression (n=5), adverse events (n=5) as well as other reasons (n=12).

2- Covariate analysis

The covariate selection was done in two steps;

I. The covariates were screened based on the visual inspection of the unexplained parameters variability-covariates relationships. Additionally, categorical covariates were evaluated using Wilcoxon signed rank test where only covariates resulting in p-value < 0.05 were further investigated for PK - SPD - leukocyte and blood pressure models. For the competing risk model, a univariate analysis was performed, where the covariate resulting in a decrease in the objective function value (OFV, i.e. -2·log likelihood) > 3.84 units (using $\chi 2$ test for df=1, α=0.05) were selected.
II. A multivariate analysis was performed in which all significant covariate relationships from the first step were included and then stepwise removed from the model. If the removal resulted in an increase in OFV of > 10.83 units (using $\chi 2$ test for df=1, α=0.001) for the PK - SPD - leukocyte and blood pressure models, and 3.84 units (using $\chi 2$ test for df=1, α=0.05) for the competing risk model, the covariate relationship was kept, otherwise it was omitted.

Only covariates with at least 25 patients per category were included. Missing categorical covariates (n ≤ 6) were imputed using the mode. The continuous covariates were log-transformed while the binary categorical covariates were expressed as 0 and 1 values.

3- Competing risk model

All patients were assigned to the state of being alive and enrolled in the study (S_1) at time = 0. Thereafter, patients were allowed to transfer to either drop-out (S_2) or death (S_3) states (i.e. absorbing states) at any time during the study period.

$$S_1(0) = 1$$

$$\frac{d(S_1)}{dt} = -\lambda_{12} * S_1 - \lambda_{13} * S_1 \qquad (eq. S1)$$

$$S_2(0) = 0$$

$$\frac{d(S_2)}{dt} = \lambda_{12} * S_1 \qquad (eq. S2)$$

$$S_3(0) = 0$$

$$\frac{d(S_3)}{dt} = \lambda_{13} * S_1 \qquad (eq. S3)$$

Here, λ_{12} is the transition rate constant from the alive to the drop-out state and λ_{13} is the transition rate constant from the alive to the dead state.

4- PK - SPD - leukocyte model

The system of ordinary differential equations (ODEs) characterizing CLL cells dynamics in lymphoid tissues and peripheral blood is given by:

$$resist = e^{-\lambda_{dec} * t}$$

$$pBTK_{eff} = pBTK_{baseline} - pBTK$$

$$CLL_{subpop,1}(0) = SPD_{baseline} * (1 - frc2) * (1 - frc1)$$

$$\frac{d\,(CLL_{subpop,1})}{dt} = k_p * (1 - slp_1 * pBTK_{eff} * resist) * CLL_{subpop,1} - k_{dtch} * (1 + slp_2 * pBTK_{eff}) * CLL_{subpop,1} \qquad (eq.\ S4)$$

$$CLL_{subpop,2}(0) = SPD_{baseline} * (1 - frc2) * frc1$$

$$\frac{d\,(CLL_{subpop,2})}{dt} = k_p * (1 - slp_1 * pBTK_{eff} * resist) * CLL_{subpop,2} - k_{dtch} * (1 + slp_3 * pBTK_{eff}) * CLL_{subpop,2} \qquad (eq.\ S5)$$

$$CLL_{subpop,3}(0) = SPD_{baseline} * frc2$$

$$\begin{aligned}\frac{d\,(CLL_{subpop,3})}{dt} = {} & k_p * (1 - slp_1 * pBTK_{eff} * resist) * CLL_{subpop,3} + k_{dtch} * (1 + slp_2 * pBTK_{eff}) * CLL_{subpop,1} + k_{dtch} \\ & * (1 + slp_3 * pBTK_{eff}) * CLL_{subpop,2} + k_h * (1 - slp_1 * pBTK_{eff}) * CLL_{bld} * SC_{cells-SPD} \\ & - (k_{dist} + k_{d,tiss} * pBTK_{eff} * resist) * CLL_{subpop,3} \qquad (eq.\ S6)\end{aligned}$$

$$CLL_{bld}(0) = CLL_{bld,baseline}$$

$$\frac{d\,(CLL_{bld})}{dt} = k_{dist} * CLL_{subpop,3} * SC_{SPD-cells} - (k_h * (1 - slp_1 * pBTK_{eff}) + k_{d,bld}) * CLL_{bld} \qquad (eq.\ S7)$$

Where, $pBTK_{baseline}$ represents the relative $pBTK$ value at baseline; $SPD_{baseline}$ represents baseline SPD; $CLL_{tiss,baseline}$ represents the total number of proliferating CLL cells in lymphoid tissues; $CLL_{bld,baseline}$ represents baseline CLL cells in peripheral blood; $frc1$ represents the fraction of $CLL_{subpop,2}$ from total CLL cells attached to the stroma at baseline; $frc2$ represents the fraction of

$CLL_{subpop,3}$ of $CLL_{tiss,baseline}$ at baseline; k_p represents the proliferation rate constant of CLL cells; k_h represents homing rate constant of CLL_{bld} from peripheral blood to lymphoid tissues; k_{dtch} represents detachment rate constant; $k_{d,bld}$ represents natural death rate constant of CLL_{bld}; $k_{d,tiss}$ represents the ibrutinib-induced death rate constant of $CLL_{subpop,3}$; slp_1 represents the slope of the ibrutinib-induced inhibitory effect on k_p and k_h; slp_2 and slp_3 represent the slopes of the ibrutinib-induced stimulatory effects on k_{dtch} for $CLL_{subpop,1}$ and $CLL_{subpop,2}$, respectively and λ_{dec} represents the exponential decay constant of ibrutinib effect over time.

5- Model development and evaluation

The first-order conditional estimation method with interaction (FOCEI) was used to estimate the PK-SPD-leukocyte and blood pressure models' parameters.

The final model selection was based on the following criteria; i) statistical significance, using $\chi 2$ test, at α=0.05, ii) the precision of the parameter estimates, and iii) graphical diagnostics.